

Increasing Freezing Tolerance: Kinase Regulation of ICE1

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Cold temperatures trigger the ICE1-CBF-COR transcriptional cascade in plants, which reprograms gene expression to increase freezing tolerance. In this issue of *Developmental Cell*, Ding et al. (2015) report that cold stress activates the protein kinase OST1 to phosphorylate and thereby stabilize and stimulate ICE1. This enhances plant tolerance to freezing temperatures.

In response to chilling temperatures, plants in temperate regions can increase their tolerance to subsequent freezing temperatures by a process known as cold acclimation. Chilling-induced reprogramming of gene expression is important for cold acclimation (Thomashow, 1999; Chinnusamy et al., 2007). Many of the cold-responsive (COR) genes have the CCGAC *cis*-element, known as the C-repeat or DRE, in their promoters. The CBFs (C-repeat-binding factors), also known as DREBs, can bind to this *cis*-element and activate the expression of COR genes (Thomashow, 1999). The three CBF genes (*CBF1*, *CBF2*, and *CBF3*) are themselves induced by cold stress, and this induction precedes that of the COR genes.

The basic-helix-loop-helix type transcription factor ICE1 binds to the *CBF3* promoter and regulates *CBF3* expression, while calmodulin-binding transcription activators (CAMTAs) bind to the *CBF2* promoter and activate *CBF2* expression in response to cold stress (Chinnusamy et al., 2003; Doherty et al., 2009). Unlike the CBFs, ICE1 and CAMTAs are expressed at warm temperatures, and the expression is not regulated by cold. Overexpression of ICE1 does not lead to CBF expression at warm temperatures but can enhance the cold induction of CBFs. Researchers have hypothesized that cold stress triggers a posttranslational modification of ICE1 that enables it to activate the CBF regulon (Chinnusamy et al., 2003). Protein phosphorylation is known to be critical for many cellular signaling pathways, and pharmacological studies indeed suggest that protein phos-

phorylation is important for cold-responsive gene regulation in plants (Chinnusamy et al., 2007). Therefore, it seems reasonable to predict that one or more protein kinases may phosphorylate ICE1 and that such phosphorylation may help ICE1 activate CBF expression.

In this issue of *Developmental Cell*, Ding et al. (2015) report that they have found such a protein kinase. The authors screened protein kinase mutants of *Arabidopsis* using a freezing tolerance assay and found that loss-of-function mutations in the protein kinase OST1 (open stomata 1), also known as SnRK2.6, reduced plant freezing tolerance. The decreased freezing tolerance was correlated with reduced cold induction of the CBF regulon. Overexpression of OST1 led to stronger cold induction of the CBF regulon and to increased freezing tolerance. Ding et al. found that OST1 physically interacts with ICE1. Importantly, they discovered that the protein kinase activity of OST1 was quickly induced by cold stress. In vitro, ICE1 could be phosphorylated by OST1 at Ser-278. In vivo, ICE1 also appeared to be phosphorylated by OST1 under cold treatment. The wild-type but not the S278A mutated form of ICE1 could restore the freezing tolerance of *ice1-2* mutant plants, indicating that phosphorylation at S278 is important for ICE1 function in planta. Phosphorylation by OST1 appeared to increase the function of ICE1 in activating the transcription of *CBF3* under cold stress.

The authors showed that OST1 also interacts with HOS1, an E3 ubiquitin ligase that interacts with and targets ICE1 for

degradation under cold stress (Dong et al., 2006). OST1 competes with ICE1 for interaction with HOS1. In addition, OST1 phosphorylation of ICE1 inhibits the interaction between ICE1 and HOS1, and the S278D mutated form of ICE1 fails to interact with HOS1. Consequently, OST1 seems to be important for ICE1 protein stability under cold stress.

The discovery of OST1 as a major protein kinase activated by cold stress is surprising. OST1 is a well-known protein kinase in the abscisic acid (ABA) signaling pathway. Under drought or salt stress, plants generate large amounts of the stress hormone ABA. ABA binds to the PYL family of receptors, changing the conformation of the receptors such that they can then interact and inhibit 2C-type protein phosphatases like ABI1 (abscisic acid insensitive 1) (Gonzalez-Guzman et al., 2012). This action releases OST1 from interaction with and inhibition by ABI1. The released OST1 is then active and can phosphorylate dozens of proteins including ion channels that help close the stomata to reduce transpirational water loss (Wang et al., 2013). Therefore, OST1 activation by ABA is well understood. But how does cold stress activate OST1? One obvious possibility is that cold stress triggers the accumulation of ABA, which in turn activates OST1. Consistent with this possibility, Ding et al. found that ABI1 inhibits not only ABA signaling but also cold stress activation of OST1. The authors also showed, however, that ABA content did not increase in plants at 0.5 and 1.0 hr after cold treatment when OST1 was activated, although there is literature reporting that

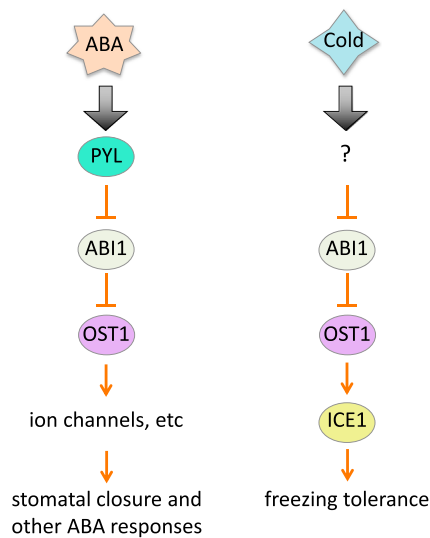


Figure 1. Comparison of Cold Stress and ABA Signaling Pathways

In the core ABA signaling pathway (left), ABA is perceived by the PYL family of receptor proteins, which then inhibit the PP2C ABI1, leading to OST1 activation. ABA-activated OST1 phosphorylates ion channels and other proteins, resulting in stomatal closure and other ABA responses. In cold stress signaling (right), the cold stress signal is perceived by an unknown sensor protein (marked by ?), which then directly or indirectly inhibits ABI1, also leading to OST1 activation. Cold-activated OST1 phosphorylates ICE1, which causes ICE1 to activate the CBF regulon, resulting in enhanced freezing tolerance.

ABA accumulates at later time points. Therefore, cold stress seems to utilize an ABA-independent mechanism to activate OST1 and inhibit ABI1 (Figure 1). Cold

temperatures may alter the conformation of a sensor protein that directly or indirectly inactivates ABI1 to activate OST1. The sensor protein could even be one or more of the PYL proteins, or even ABI1 itself. Structural studies suggest that ABA-independent functions are possible for some of the PYL proteins (Hao et al., 2011). It would be interesting to test how *pyl* mutant plants respond to cold stress. For example, a sextuple mutant defective in six PYL ABA receptors is extremely insensitive to ABA (Gonzalez-Guzman et al., 2012). Is OST1 activation affected in this and other high-order *pyl* mutants?

The discovery by Ding et al. of a cold-stress-activated protein kinase that phosphorylates ICE1 and controls the CBF regulon is an important advancement in our understanding of cold stress signaling in plants. The research raises interesting questions regarding possible cold stress sensors and how cold sensing leads to the activation of OST1. Additionally, how do we explain that, although cold activates OST1, the physiological responses to cold stress are largely different from those of ABA (Figure 1)? Clearly, factors besides OST1 are also important. In this regard, it would be especially helpful to place the findings of Ding et al. in the context of previous work showing that a mitogen-activated protein kinase pathway and a calcium/calmodulin-dependent protein kinase regulate the

CBF regulon and freezing tolerance (Teige et al., 2004; Yang et al., 2010).

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